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Embryo microinjection and mutation screening

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An abbreviated version of this protocol was published in eLIFE in Jan 2020

Development of a confinable gene drive system in the human disease vector *Aedes aegypti*

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Detailed protocol

Embryonic collection and CRISPR microinjections were performed following previously established procedures (Aryan et al., 2014; Kistler et al., 2015). The concentration of plasmids used for the U6 promoter screen was 300 ng/μL. Injected G_0 and G_1 progeny were visualized at the larval, pupal, and adult life stages under a dissecting microscope (Olympus SZ51 and Leica M165FC). The heritable mutation rates were calculated as the number of G_1 progeny with the loss-of-function mutation out of the number of all G_1 progeny crossed with the white eye (*w*–) strain mosquitoes. To integrate each GDe construct at the *white* locus, a mixture containing 100 ng/μL of synthetic gRNA^{w}, 100 ng/μL of each U6-GDe plasmid (U6a-GDe, U6b-GDe, U6c-GDe, and U6d-GDe), and 100 ng/μL of Cas9 protein was injected into 500 *w*‒ embryos for each plasmid. Synthetic gRNAs (Synthego) and recombinant *Streptococcus pyogenes* Cas9 protein (PNA Bio Inc, Supplementary file 8a) were obtained commercially and diluted to 1,000 ng/μL in nuclease-free water and stored in aliquots at –80°C. A total of 233, 271, 191, 215 G_0 adults were recovered for U6a-, U6b-, U6c-, and U6d-GDe injections, respectively. Successful integration into the *white* locus was determined by visually identifying the eye-specific 3xP3-tdTomato fluorescence in G_1 heterozygous mosquito larvae with black eyes ($w^{U6\text{-}GDe}/w^{+}$) and in G_2 homozygous mosquito larvae with white eyes ($w^{U6\text{-}GDe}/w^{U6\text{-}GDe}$). In addition, site-specific integration of U6-GDe constructs was confirmed by amplifying and Sanger sequencing both the left and right integration points (Figure 2—figure supplement 2) from a genomic DNA prep of each $w^{U6\text{-}GDe}$ line with the following primers: AE20, AE21, AE22, and AE23 (Supplementary file 8b).

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Akbari, O. S. and Li, M. (2022). Embryo microinjection and mutation screening. Bio-protocol Preprint. bio-protocol.org/prep1833.
2. Li, M., Yang, T., Kandul, N. P., Bui, M., Gamez, S., Raban, R., Bennett, J., Sánchez C, H. M., Lanzaro, G. C., Schmidt, H., Lee, Y., Marshall, J. M. and Akbari, O. S.(2020). Development of a confinable gene drive system in the human disease vector *Aedes aegypti*. eLIFE. DOI: 10.7554/eLife.51701